A4

64. The method of Claim 63, wherein the alternative splicing regulatory protein is selected from the group consisting of hnRNP A/B protein, hnRNP A protein, hnRNP B protein, and hnRNP A1 protein.

These amendments and new claims do not include new matter. Support for the amendment and the new claims can at least be found on pages 21, 30, 40 and 41. Additionally, new Claim 55 recites an appropriate alternative limitation. MPEP § 2173.05(h) (8th ed. Rev. 1 2003).

REMARKS

Claims 1-64 are pending in this case. Claims 31-36 and 43-54 are withdrawn from further consideration without prejudice. Claims 21, 22, and 37-41 are objected to and Claims 1-30 and 37-42 are rejected. Claims 16-20, 22-28 and 37-42 are canceled without prejudice. Claims 1-15, 21, and 29-30 are amended. New Claims 55-64 are added. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. CLAIM OBJECTIONS

The Office objected to Claims 37-42 as being substantial duplicates of previous claims. Claims 37-42 have been canceled without prejudice and therefore, obviates this objection.

The Office also objected to Claims 21 and 22 under 37 C.F.R. § 1.75(c), as being improper dependent form for failing to further limit the subject matter of a previous claim. Claim 21 is amended and further limits the subject matter from which it depends by including an additional step. Claim 22 was canceled and therefore, obviates this objection.

II. CLAIM REJECTIONS - 35 U.S.C. § 112

A. CLAIMS 1-28 AND 37-42 ARE DEFINITE UNDER 35 U.S.C. § 112, SECOND PARAGRAPH.

Claims 1-28 and 37-42 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is traversed.

Claims 16-20, 22-28 and 37-42 have been canceled without prejudice and therefore, obviates the rejection with respect to these claims.

With regard to Claims 1-15 and 21, Claim 1 was amended to particularly point out and distinctly claim the subject matter which Applicant regards as an embodiment of the invention. Since Claims 1-15 and 21 depend from.and contain all the limitations of Claim 1, Claims 1-15 and 21 also point out and distinctly claim the subject matter which Applicant regards as an embodiment of the invention in the same manner as Claim 1. In addition, Claims 2-15 and 21were amended.

In particular, the Office states that "Claim 1 recites 'the polynucleotide sequences' [line] step b) but it is not clear what the metes and bounds are for the limitation." As stated *supra*, Claim 1 was amended to further recite the metes and bounds of the claim.

The Office also states that "Claims 1, 23, 27, and 37 recite the limitation 'the activity' in line 1. There is insufficient antecedent basis for this limitation in the claim." Amended Claim 1 rectifies the antecedent basis issue.

Finally, the Office states that "claim 14 recites 'the polynucleotide sequences are single-stranded' but it is not clear what the metes and bound[s] are for the limitation. Does claim 14 further limit[ ] step a) or step b) of claim 1." Amended Claim 14 further identifies the metes and bounds of the limitation and addresses the step of the method it limits.

B. CLAIMS 1-28 AND 37-41 ARE ENABLED UNDER 35 U.S.C. § 112, FIRST PARAGRAPH.

Claims 1-28 and 37-41 were rejected under 35 U.S.C. § 112, first paragraph. The Office states that the specification, while being enabling for a method of modifying activity of hnRNP A proteins, does not reasonably provide enablement for a method of modifying activity of any other nucleotide binding proteins. This rejection is traversed.

Claims 16-20, 22-28 and 37-42 have been canceled and therefore, obviates a part of this rejection. With regard to Claims 1-15 and 21, Claim 1 was amended based on the specification that describes how to make and how to use an embodiment of the invention. Since Claims 1-15 and 21 depend from and contain all the limitations of

Claim 1, Claims 1-15 and 21 also were amended based on the specification in the same manner as Claim 1.

In particular, the Office states that "Claim 5 is rejected ... as drawn to cell transfection method using a detergent since claims 2-5 appear[ ] to be drawn to transfection of cells with a polynucleotide. Neither the specification nor the art teaches transfection method using a detergent." The use of a detergent for transfection is commonly known in the art. For instance, it is known that detergents disrupt hydrophobic associations and destroy the lipid bilayer. *See* Bruce Alberts, *et al.* THE CELL 488-89 (3rd ed. 1994). Essentially, detergents separate integral proteins bound to membranes creating a permeable membrane allowing for transfection. *See* Donald Voet and Judith G. Voet, BIOCHEMISTRY 291-92 (2d ed. 1995).

In an embodiment, the specification provides transfection by electroporation. Transfection methods are well known in the art and may include electroportation and detergent treatments and any other method appropriate for transfection. Detailed procedures for making and using the invention are not necessary if the description of the invention itself is sufficient to permit those skilled in the art to make and use the invention. MANUAL OF PATENT EXAMINING PROCEDURE § 2164 (8th ed. Rev. 1 2003) (hereinafter "MPEP"). The level of one of ordinary skill is such that one in the art would understand transfection as including many techniques, i.e., but not limited to, electroporation and/or detergent treatment.

Further, the Office states that "Claim 13 is rejected ... as drawn to method of modifying an activity of a nucleotide binding proteins in cells by transfecting a RNA

analog. Neither the specification nor the art teaches any RNA analog capable of modifying an activity of a nucleotide binding protein." RNA analogs are commonly used in the art. The level of one of ordinary skill is such that one of ordinary skill would understand a RNA analog used in transfection. Detailed procedures for making and using the invention are not necessary if the description of the invention itself is sufficient to permit those skilled in the art to make and use the invention. MPEP § 2164.

Additionally, the Office states that "Claim 19 is rejected ... as drawn to method of modifying an activity of a nucleotide binding protein by binding of the protein to a transfected polynucleotide irreversibly." Claim 19 was canceled and therefore, obviates this rejection.

III. CLAIM REJECTIONS - 35 U.S.C. § 102

A. CLAIMS 1, 3, 6, 11, 15-18, 20, 22-30, AND 37-42 ARE PATENTABLY DISTINGUISHABLE OVER BLANCHETTE, *ET AL.*

Claims 1, 3, 6, 11, 15-18, 20, 22-30, and 37-42 were rejected under 35 U.S.C. § 102(b) as being anticipated by Blanchette, *et al.* This rejection is respectfully traversed.

The Office states that Blanchette, *et al.* teaches "[a] method of modifying activity (Exon skipping) of an hnRNP A1 protein in HeLa cells (human tissue culture cells) by transfecting a minigene (shown in Fig. 2 at page 1941) with the hnRNP binding sites and Northern analysis detection of the activity of the protein."

In particular, Blanchette introduced into HeLa cells a genomic portion of the mouse hnRNP A1 alternative splicing unit as part of a minigene. Blanchette at 1940. In doing so, Blanchette attempts to determine whether the mouse *cis*-acting sequences, physically *linked* to a target RNA sequence, CE1 and CE4 control the inclusion of exon 7B in the splice RNA specified by the minigene. The success of Blanchette's experiments depends on the cellular protein recognizing and binding to the *cis*-acting CE1 and CE4 elements. Thus, Blanchette generally shows RNA sequences acting in *cis* to influence splicing of a transcript. Applicants' invention, however, is patently distinguishable from Blanchette.

Applicants' invention uses a polynucleotide sequence in *trans*, physically *unlinked* to a target RNA sequence, as disclosed and claimed. In an embodiment, Fig. 5a illustrates chicken leg bud mesenchymal cells transfected with FGFR2 exon 9 sense strand RNA and exon 8 sense strand RNA. *See* Application at 67. Notably absent are various carrier (i.e., the physical linkage to a target RNA sequence). Instead, these polynucleotide sequences are introduced into cells in a free or soluble form. The polynucleotide sequences bind to the hnRNP A1 binding protein and therefore, compete with the protein's binding to, for instance, endogenous RNA sequences (ISS, ISE, ESS and/or ESE).

Further, Blanchette's experiments demonstrate whether the minigene could recognize and bind to the CE1 and CE4 elements. Blanchette's experiments and disclosure do not indicate a method of regulation or competition, as recited by Applicants' invention. Thus, Applicants' invention is patentably distinguishable from the

disclosure of Blanchette, *et al.* In addition, Claims 16-18, 20, and 22-28 have been canceled and therefore, obviates a part of this rejection.

B. CLAIMS 1, 3, 6-8, 10-12, 14-18, 20, 22, AND 37-40 ARE PATENTABLY DISTINGUISHABLE OVER MCNALLY, *ET AL.*

Claims 1, 3, 6-8, 10-12, 14-18, 20, 22, and 37-40 were rejected under 35 U.S.C. § 102(b) as being anticipated by McNally, *et al.* This rejection is respectfully traversed.

The Office states that McNally, *et al.* "teach[es] a method of modifying snRNP proteins by transfecting a plasmid derived from Rous sarcoma virus."

In particular, McNally examines the *cis*-acting negative regulator of splicing (NRS) in Rous sarcoma virus. McNally at 2385 (Abstract). NRS was found to bind several cellular RNA proteins involved in the splicing machinery. *Id.* at 2385. McNally studied mutations in NRS that disrupted binding to one or another RNA binding protein. *Id.* at 2386. McNally concluded that NRS-mediated splicing inhibition includes the small nuclear ribonuclear protein U1. *Id.* at 2387.

In Figs. 1-6, McNally demonstrates the effects of modifications of *cis*-acting elements on the splicing of the minigene. The experiments in Fig. 7 used affinity selection to show that U1 can bind to the NRS. Applicants invention, however, is patentably distinguishable from that discloses in McNally, *et al.*

Applicants' invention uses a polynucleotide sequence in *trans*, physically *unlinked* to a target RNA sequence, as disclosed and claimed. In an emboidment, Fig. 5a illustrates chicken leg bud mesenchymal cells transfected with FGFR2 exon 9 sense

strand RNA and exon 8 sense strand RNA. *See* Application at 67. These polynucleotide sequences are introduced into cells in a free or soluble form. The polynucleotide sequences bind to the RNA binding protein and therefore, compete with the protein's binding to, for instance, endogenous RNA sequences (ISS, ISE, ESS and/or ESE).

Further, McNally modifies a domain integral to an intact protein that influences the proteins function. Applicant's invention, however, does not modify a domain integral to an intact protein. Thus, Applicants' invention is patentably distinguishable from the disclosure of McNally, *et al.* In addition, Claims 16-18, 20, 22 and 37-40 have been canceled and therefore, obviates a part of this rejection.

C. CLAIMS 1-4, 6, 14, 15, 18, 20, 21, AND 37-39 ARE PATENTABLY DISTINGUISHABLE OVER CACERES, *ET AL.*

Claims 1-4, 6, 14, 15, 18, 20, 21, and 37-39 were rejected under 35 U.S.C. § 102(b) as being anticipated by Caceres, *et al.* This rejection is respectfully traversed.

The Office states that Caceres, *et al.* teaches "transfection methods under [c]ell culture ..., and the transfected polynucleotide modifying the activity of a nucleotide binding protein[ ] such as RNA and DNA polymerase, many transcription factors and translation factors (all nucleotide binding proteins either double or single stranded RNA or DNA) [are] inherent consequences of the transfection."

Caceres introduces "polynucleotides," i.e., plasmids, encoding normal and mutant forms of various RNA binding proteins into cells. Caceres at 56, 61. The effect of these plasmids is to direct the production of these proteins and to determine how

mutation or swapping of different protein domains affected the nuclear transport and nuclear-cytoplasmic shuttling of the proteins. *Id.* at 62-63. McNally failed to disclose or utilize polynucleotides as competitors of endogenuous RNA substrates by the RNA binding proteins as Applicants' invention discloses and claims. Generally, the Caceres reference discloses that the modification or addition of a domain integral to an intact protein influences that protein's function.

Further, Caceres does not report that a polynucleotide was used as a *trans*-acting agent to influence activity of an RNA binding protein. Thus, Applicants' invention is patentably distinguishable from Caceres, *et al.* In addition, Claims 18, 20 and 37-39 have been canceled and therefore, obviates a part of this rejection.

IV. CLAIM REJECTIONS - 35 U.S.C. § 103

A. CLAIM 9 IS PATENTABLY DISTINGUISHABLE OVER BLANCHETTE, *ET AL.* IN VIEW OF ROSS, ET AL.

Claim 9 was rejected under 35 U.S.C. § 103(a) as being unpatentable over Blanchette, *et al.* in view of Ross, *et al.* This rejection is respectfully traversed.

The Office states that "Ross, *et al.* teach[es] avian cells have nucleotide binding proteins. Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to transfect avian cells with a polynucleotide for modifying the activity of the nucleotide binding protein with a reasonable expectation

of success." However, in order to establish a *prima facie* showing of obviousness under Section 103, the examiner must set forth three basic criteria.

First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the references or to combine the reference teachings. Second, there must be a reasonable expectation of success in the combination. Finally, the prior art references must teach or suggest all the claim limitations. *See* MPEP §§ 706.02(j), 2142 (8th ed. 2001).

In this instance, the Office failed to establish a *prima facie* case of obviousness. Particularly, the Office failed to provide a suggestion or motivation for combination and failed to indicate why there is a reasonable expectation of success in the combination. The mere conclusion that there is "a reasonable expectation of success" does not satisfy this burden. The Federal Circuit recently explained that the motivation or suggestion to combine the references "is material to patentability, and could not be resolved on subjective belief and unknown authority." *In re Lee*, 61 U.S.P.Q.2d (BNA) 1430, 1434 (Fed. Cir. 2002). "Thus, when they [the Board and/or the Office] rely on what they assert to be general knowledge to negate patentability, that knowledge must be articulated and placed on the record." *Id.* at 1435.

Blanchette, *et al.*, as stated *supra*, introduced into HeLa cells a genomic portion of the mouse hnRNP A1 alternative splicing unit as part of a minigene. Blanchette sought to determine whether the mouse *cis*-acting sequences CE1 and CE4, located upstream and downstream of mouse hnRNPA1 exon 7B controlled in the inclusion of

exon 7B in the spliced RNA specified by the minigene. Blanchette's experiments depended on the cellular protein recognizing and binding with *cis*-acting elements in order to influence splicing or change the cellular phenotype in any manner.

Because Blanchette's disclosures and methods do not teach the use of a *trans*-acting polynucleotide to alter the activity of RNA binding protein, the combination of Blanchette, *et al.* and Ross, *et al.* does not suggest Applicants' invention nor provide for a reasonable expectation of success in the combination. Further, the prior art references do not teach or suggest all the claim limitations. Thus, Applicants' invention is patentably distinguishable over Blanchette, *et al.* in view of Ross, *et al.*

Claim 9 depends from Claim 1. Because of this dependency, Claim 9 is construed to incorporate all of the limitations of Claim 1. *See* 35 U.S.C. § 112 (1994). As stated under the Section 102 analysis, Claim 1 is patentably distinguishable from the disclosures in Blanchette, *et al.* and thus, Claim 9 is thought to include all the limitations of Claim 1 and to provide an additional limitation, namely that the cells are avian cells. Blanchette in view of Ross does not teach all of the claim limitations and thus, Claim 9 is patentably distinguishable.

V. CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully submit that the claims of the present invention define subject matter patentable over the references cited by the Examiner and that the application is in condition for allowance. Should the Examiner believe that anything further is desirable to place the application in

better condition for allowance, the Examiner is invited to contact Applicants' undersigned attorney at the below listed telephone number.

The Commissioner is hereby authorized to charge any deficiency or credit any overpayment to deposit account number 03-2469. Moreover, if the deposit account contains insufficient funds, the Commissioner is hereby invited to contact Applicant's undersigned representative to arrange payment.

Respectfully submitted,

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MARKED-UP VERSION OF AMENDMENT AND RESPONSE

IN THE CLAIMS:

1. (ONCE AMENDED) A method of modifying [the] an activity of at least one alternative splicing regulatory protein [nucleotide binding proteins] within at least one cell[s] comprising:

[a)]introducing into the cell[s] a plurality of polynucleotide sequences directed to the alternative splicing regulatory protein [nucleotide binding proteins]; and

[b)]interacting [binding within cells the polynucleotide sequences to the nucleotide binding proteins] the polynucleotide sequences to the alternative splicing regulatory protein within the cell, wherein the polynucleotide sequences compete with at least one endogenous RNA sequence for interacting with the alternative splicing regulatory protein. [; and

c) modifying within cells the activity of the nucleotide binding proteins with said binding.]

2. (ONCE AMENDED) The method [according to] of Claim 1, wherein the polynucleotide sequences are introduced into the cell[s] by electroporation.

3. (ONCE AMENDED) The method [according to] of Claim 1, wherein the polynucleotide sequences are introduced into the cell[s] by applying the polynucleotide sequences to the surface of the cell[s].

4. (ONCE AMENDED) The method [according to] of Claim 3, wherein the polynucleotide sequences are packaged in at least one liposome.

5. (ONCE AMENDED) The method [according to] of Claim 3, wherein the polynucleotide sequences are applied to a [the] surface of the cell[s] along with a detergent.

6. (ONCE AMENDED) The method [according to] of Claim 1, wherein the cell[s are] is at least one tissue culture cell[s].

7. (ONCE AMENDED) The method [according to] of Claim 1, wherein the cell[s are] is at least one non-human cell[s].

8. (ONCE AMENDED) The method [according to] of Claim 1, wherein the cell[s are] is at least one non-human mammalian cell[s].

9. (ONCE AMENDED) The method [according to] of Claim 1, wherein the cell[s are] is at least one avian cell[s].

10. (ONCE AMENDED) The method [according to] of Claim 1, wherein the cell[s are] is at least one non-human tissue culture cell[s].

11. (ONCE AMENDED) The method [according to] of Claim 1, wherein the polynucleotide sequences further comprise at least one isolated and purified RNA molecule[s].

12. (ONCE AMENDED) The method [according to] of Claim 1, wherein the polynucleotide sequences further comprises at least one synthetic RNA molecule[s].

13. (ONCE AMENDED) The method [according to] of Claim 1, wherein the polynucleotide sequences further comprise at least one synthetic RNA analog[s].

14. (ONCE AMENDED) The method [according to] of Claim 1, wherein the polynucleotide sequences are single-stranded.

15. (ONCE AMENDED) The method [according to] of Claim 1, wherein the step of [modifying within cells the activity of the nucleotide binding proteins] interacting the polynucleotide sequences to the alternative splicing regulatory protein further comprises regulating the activity of the alternative splicing regulatory protein [nucleotide binding proteins].

21. (ONCE AMENDED) The method [according to] of Claim 1, further comprising the step of determining [the] an effect [in the processing of RNA] on RNA processing by at least one [the] resulting phenotypic characteristic[s] of the cell[s].

29. (ONCE AMENDED) A method of modifying [the] an activity of at least one hnRNP A1 protein[s] within at least one cell[s] comprising:

[a]]introducing into the cell[s] a plurality of sequences capable of binding to the hnRNP A1 protein; and

[b] binding within cells]interacting the polynucleotide sequences to the hnRNP A1 protein[s] within the cell, wherein the polynucleotide sequences compete with at least one endogenous RNA sequence for interacting with the hnRNP A1 protein [;and

c) modifying within the cells the activity of the hnRNP A1 proteins with said binding].

30. (ONCE AMENDED) The method [according to] of Claim 29, further comprising the step of [causing] determining an effect [within cells in the processing of RNA] on RNA processing by [modifying the activity of the hnRNAP A1 proteins] at least one resulting phenotypic characteristic of the cell.

55. (NEW) The method of Claim 1, wherein the polynucleotide sequences comprise a plurality of sequences from an mRNA wherein the sequences from the mRNA are intronic splicing silencers, intronic splicing enhancers, exonic splicing silencers or exonic splicing enhancers.

56. (NEW) The method of Claim 1, wherein the alternative splicing regulatory protein is selected from the group consisting of hnRNP A/B protein, hnRNP A protein, hnRNP B protein, and hnRNP A1 protein.

57. (NEW) A method of modifying an activity of at least one alternative splicing regulatory protein within at least one cell, which comprises the steps of:

introducing into the cell a plurality of polynucleotide sequences comprising at least one intronic splicing silencer; and

interacting the polynucleotide sequences to the alternative splicing regulatory protein within the cell, wherein the polynucleotide sequences compete with at least one endogenous RNA sequence for interacting with the alternative splicing regulatory protein.

58. (NEW) The method of Claim 57, wherein the alternative splicing regulatory protein is selected from the group consisting of hnRNP A/B protein, hnRNP A protein, hnRNP B protein, and hnRNP A1 protein.

59. (NEW) A method of modifying an activity of at least one alternative splicing regulatory protein within at least one cell, which comprises the steps of:

introducing into the cell a plurality of polynucleotide sequences comprising at least one intronic splicing enhancer; and

interacting the polynucleotide sequences to the alternative splicing regulatory protein within the cell, wherein the polynucleotide sequences compete with at least one endogenous RNA sequence for interacting with the alternative splicing regulatory protein.

60. (NEW) The method of Claim 59, wherein the alternative splicing regulatory protein is selected from the group consisting of hnRNP A/B protein, hnRNP A protein, hnRNP B protein, and hnRNP A1 protein.

61. (NEW) A method of modifying an activity of at least one alternative splicing regulatory protein within at least one cell, which comprises the steps of:

introducing into the cell a plurality of polynucleotide sequences comprising at least one exonic splicing silencer; and

interacting the polynucleotide sequences to the alternative splicing regulatory protein within the cell, wherein the polynucleotide sequences compete with at least one

endogenous RNA sequence for interacting with the alternative splicing regulatory protein.

62. (NEW) The method of Claim 61, wherein the alternative splicing regulatory protein is selected from the group consisting of hnRNP A/B protein, hnRNP A protein, hnRNP B protein, and hnRNP A1 protein..

63. (NEW) A method of modifying an activity of at least one alternative splicing regulatory protein within at least one cell, which comprises the steps of:

introducing into the cell a plurality of polynucleotide sequences comprising at least one exonic splicing enhancer; and

interacting the polynucleotide sequences to the alternative splicing regulatory protein within the cell, wherein the polynucleotide sequences compete with at least one endogenous RNA sequence for interacting with the alternative splicing regulatory protein.

64. (NEW) The method of Claim 63, wherein the alternative splicing regulatory protein is selected from the group consisting of hnRNP A/B protein, hnRNP A protein, hnRNP B protein, and hnRNP A1 protein.